Feasibility of multi-lectin glycoprotein capture combined with free-flow electrophoresis (FFE) separation prior to LC-MS/MS bottom-up identification of plasma glycoproteins

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Overview

Investigation of the feasibility of an analysis approach combining multi-lectin glycoprotein capture and free-flow electrophoresis (FFE) prior to high resolution LC-MS/MS bottom-up analysis of human plasma glycoproteins.

Introduction

- **The mission of proteomics** is to reveal proteins as potential early biomarkers in human diseases. **Glycosylation** is the most abundant post-translational modification affecting roughly 50 % of the proteome, potential **biomarkers** will therefore likely be glycoproteins.
- **Plasma** is an easily accessible body fluid, it can reflect the human health status, but is also a very complex type of sample with thousands of proteins in a concentration range exceeding 10 orders of magnitude. This **sample complexity** can be addressed by combining extensive sample preparation steps (e.g. protein depletion, 2D-gel, LC) prior to the LC-MS/MS analysis.
- **Major limitations** of these laborious steps are sample throughput, dynamic range and loading capacity. Glyco- proteome studies identified some 10–600 glycoproteins so far (1-5) with the number of protein assignments correlated with the extent of sample preparation.
- **Glycoprotein capture** can significantly reduce the sample complexity by focusing on the glycosylated sub-proteome. In contrast to methods using a covalent binding, lectin affinity based methods offer a non-destructive separation which keeps the whole glycoprotein information intact (6-8).
- **Free-flow electrophoresis** is a liquid separation method based on the electrophoretic ion mobility. Its orthogonal separation principle, the continuous mode of operation and the excellent separation power make it an interesting tool for protein separation prior to LC-MS/MS analysis (9-11).

Methods

Glycoprotein capture was investigated using immobilized lectins (ConA and WGA) from Pierce (Rockford, US). We used the FFE enhanced system from BD & Company (formerly Dr. Weber GmbH, Germany) for isoelectric focusing with HPMC/Urea protocols according to the manufacturers procedure. A scheme of the separation principle is given in Figure 1.

Bottom-up protein analysis was carried out after reduction, alkylation and enzymatic digestion (PNGaseF and trypsin) using a high-mass-resolution LC-MS/MS system (LTQ-FT-ICR). Indicative peptides were searched against the human Swissprot database using Mascot, results were categorized using DBParser (12).

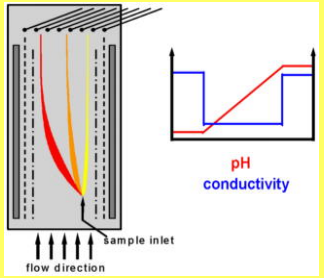

Figure 1: Scheme of the FFE separation principle (BD & Company)

Literature

(1) Yang et al. J Chrom A 2004
(2) Yang et al. Proteomics 2005
(3) Liu et al. J Proteome Res 2005
(4) Wang et al. Mol Cell Proteomics 2006
(5) Liu et al. Mol Cell Proteomics 2006
(6) Haegglund et al. J Proteome Res. 2003
(7) Wada et al. Anal Chem 2004
(8) Zhang et al. Nat Biotechnol 2003
(9) Weber & Bocek Electrophoresis 1998
(10) Moritz et al. Anal Chem 2004
(11) Xie et al. Anal Chem 2005
(12) Yang et al. J Proteome Res 2004

Results – Multi-lectin glycoprotein capture

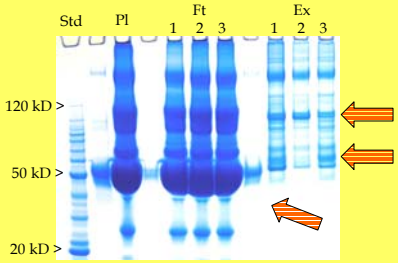

Figure 2: Coomassie blue stained gradient gel of human plasma (Pl), flow through (Ft) and extract (Ex) of lectin affinity spin columns (1-Mixture of ConA and WGA lectin, 2-WGA lectin only, 3-ConA lectin only)

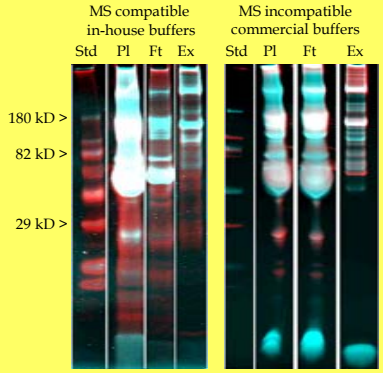

Figure 3: Gradient gel of human plasma (Pl), flow through (Ft) and extract (Ex) of a multi-lectin affinity spin column. Red channel – Sypro Ruby protein stain, green/blue channel – Emerald Pro 488 glyco-protein stain.

Results – Free-flow electrophoresis (FFE) separation

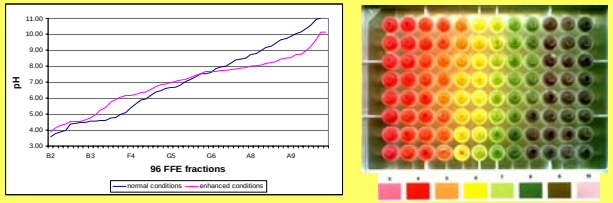

Figure 4: pH-value distribution in the 96-wells of a FFE separation measured by pH microelectrode or simply by adding a universal pH indicator.

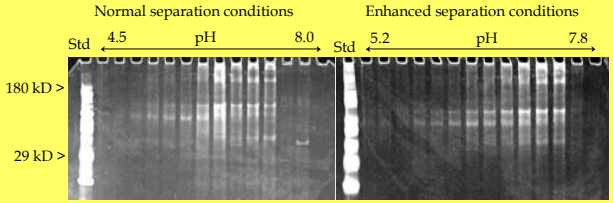

Figure 5: Gradient gel of selected FFE fractions after separation of a glycoprotein enriched human plasma sample in both a normal and an enhanced separation mode (HPMC/Urea protocol, Sypro Ruby protein staining).

Results – LC-MS/MS bottom-up glycoprotein identification

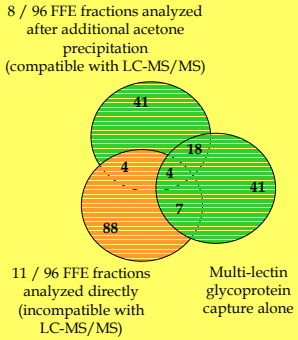

Figure 6: Number of distinct protein assignments (DBParser analysis) after different steps of sample preparation of human plasma.

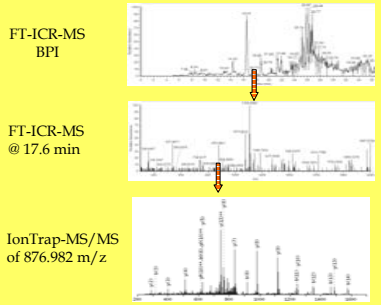

Figure 7: Analysis example of the FFE fraction 4A. MS/MS characterization of the peptide (YVGGQEHFAHLLILR) resulted in a distinct glycoprotein assignment of alpha- 1-acid glyco-protein 1-precursor, AGP 1 (OMD 1).

Conclusions

- Lectin based glycoprotein capture can greatly enrich glycoproteins and can deplete otherwise over-shadowing proteins within the same sample preparation step.
- A multi-lectin approach can significantly broaden the glycoprotein specificity, a prerequisite for glyco-proteome studies, but more than two lectins are needed to completely cover this sub-proteome.
- Commercial lectin affinity kits contain detergents (e.g. CHAPS) that are incompatible with subsequent LC-MS/MS analysis. Buffers can successfully be substituted by in-house buffers, which proved to be of similar glycoprotein capture quality.
- FFE separation is an orthogonal and very powerful separation tool ($\Delta pI<0.1$), but FFE buffer composition can be problematic for downstream LC MS/MS analysis. Acetone precipitation can solve this compatibility issue on the extend of analytical sensitivity.

➢ Major challenges so far are the incomplete plasma glycoprotein enrichment and the LC-MS/MS incompatible FFE buffers, which are the major reason for the presently rather limited number of glycoprotein assignments (~100) possible with this analytical approach towards the human plasma glycoproteome.

Acknowledgments

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